Towards small and fast size-exclusion chromatography
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Chapter 3
Towards Miniaturized Size-Exclusion Chromatography

Abstract
Miniaturized size-exclusion chromatography has a number of important advantages, such as the use of greatly reduced amounts of solvents and samples. However, miniaturized SEC has received very little attention in the literature so far.
In this chapter a number of aspects of miniaturized SEC are considered. Chromatographic band-broadening phenomena are shown to be critical. The calibration curves and molar-mass distributions obtained using columns with different internal diameter are statistically compared.

3.1. Introduction
Size-Exclusion Chromatography (SEC) is the outstanding technique for measuring molar-mass distributions (MMDs) of natural and synthetic macromolecules. Since its introduction in 1959 by Porath and Flodin [1] as gel-permeation chromatography (GPC), when the stationary phase was presented as "lightly cross-linked, semi-rigid, organic-polymer networks", SEC developed enormously. It became one of the most robust methods in liquid chromatography (LC). As described in chapter 1, SEC can be coupled with various detectors to provide information on polymeric samples [2,3,4,5,6,7,9]. Several improvements of SEC, are, however, greatly needed. In areas such as forensic science [8], art conservation [9], and systems biology there is a very high demand for miniaturized techniques. In these fields only very small amounts of sample are available. For example, in art conservation paper ageing can be investigated by measuring, among others properties, bursting strength [10], folding endurance [11], and tensile breaking [12]. To perform measurements of these properties on valuable drawings or documents would require exorbitantly large sample sizes. Therefore, methods able to analyze very small amounts of sample are indispensable. Using SEC, the degradation of the cellulose chains can be estimated by comparing the MMD of (artificially) aged and new samples [13,14]. Using advanced, but not very robust miniaturized separation methods, such as size-exclusion
electrochromatography (SEEC), repeated analysis could be performed on a single paper fiber [15].

There are many good incentives to explore robust, miniaturized SEC methods for use in routine (industrial) practice. The smaller the column volume, the less stationary and mobile phase will be required and the less toxic waste will be produced [16]. This will reduce costs and make the technique more environment-friendly. The use of miniaturized systems may – eventually – make it easier to adequately control the temperature in the analysis of polymers that cannot be dissolved at room temperature (e.g. polyolefins [17]). The compatibility of SEC with certain detectors (e.g. mass-spectrometry) will be enhanced. Nielsen et al. showed that hyphenation of SEC (on-line) with electrospray-ionization mass spectrometry (ESI-MS) [18] and (off-line) with matrix-assisted laser-desorption/ionization (MALDI) MS [19] can be used very elegantly for accurate molar-mass calibration and for polymer characterization. Miniaturized SEC columns are desirable if SEC is to be used as first-dimension separation in comprehensive two-dimensional liquid chromatography (i.e. SEC×LC). Cortes et al. quantitatively determined polymer additives by on-line coupling of micro-size-exclusion chromatography and liquid chromatography (μSEC-LC) and gas chromatography (μSEC-GC) [20]. Blomberg et al. [21] described fully automated analysis of low-MM materials in polymers using μSEC-GC. Even with miniaturized SEC columns the volumes of sample that must be introduced in the GC are quite large, so that miniaturization is an absolute necessity for the on-line coupling of SEC with GC. If the sole purpose of the hyphenation is sample clean-up (separating the polymer from low-MM additives, monomers, etc.), then the demands on the resolution of the SEC system are relatively low.

Among the greatest obstacles encountered when trying to realize polymer analysis by miniaturized SEC are those caused by the slow diffusion of the analytes. This leads to increased dispersion within and outside the column. Therefore highly efficient (very well packed) columns, miniaturized connections and injectors are essential. Unfortunately, miniaturized SEC columns are not yet commercially available, which is definitely holding back the development of the technique. Commercially available detectors that are compatible with miniaturized systems include UV, MS [16,20], and fluorescence detectors [22]. Several other important SEC detectors (refractive index, viscometry, light scattering) cannot currently be used. The reduced variety of available detection systems is another obstacle to the miniaturization of SEC. SEEC poses less stringent requirements on the
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quality of the column packing. The flat flow profile inside the column will lead to more homogeneous velocities within the sample zone [23,24]. However, none of the existing injection systems and hardly any of the detectors used in SEC can be used in SEEC.

An alternative to the use of conventional packed columns in miniaturized SEC is the use of monolithic columns. Monolithic columns have been developing rapidly during the last few years [25,26,27]. They provide a unique combination of a low-pressure drop and high separation efficiency. Unfortunately, the selectivity offered by monolithic columns for SEC is still much inferior to that of typical columns packed with porous particles. So far, monoliths have a smaller volume of pores that contain stagnant mobile phase during the analysis.

In summary, the main requirements for achieving high-performance miniaturized SEC are very-well-packed columns, very-well-designed instruments, and miniaturized detectors. The objectives of this work are to investigate and understand the effects of decreasing the diameter of the separation columns to micro-bore dimensions (viz. 1 mm i.d.) and to realize miniaturized SEC separations.

3.2. Theory

Conventionally, SEC is performed in (series of) columns with (combined) lengths of 500 to 1500 mm and internal diameters (i.d.) of 7.6 to 10 mm. Such columns typically require a minimum amount of 40 μL sample solution. The volume of mobile phase required per analysis varies from about 20 to 100 ml, which at a typical flow rate of 1 ml/min corresponds to analysis times of 20 to 100 min. A comparison of columns of different dimensions is provided in Table 1. Decreasing the size of separation columns by reducing the column diameter has numerous advantages, as described in the introduction of this chapter.

The target in miniaturization of SEC is to obtain identical chromatograms (same peak widths and eventually the same MMDs), while injecting less sample and using less eluent (implicitly producing less toxic waste).
The injection volume and the flow rate must be reduced in order to keep the loading of the system and the transversal velocity inside the column constant. In practice the adjustment of the flow rate (eq. 1) and injection volume (eq. 2) is as follows

\[
F_2 = \left( \frac{d_{c,2}}{d_{c,1}} \right)^2 \times F_1
\]  
(1)

\[
V_{inj,2} = \left( \frac{d_{c,2}}{d_{c,1}} \right)^2 \times V_{inj,1}
\]  
(2)

where \( F_1 \) and \( F_2 \) are the flow rates of the first and second separation column, respectively, \( d_{c,1} \) and \( d_{c,2} \) are the respective column diameters. \( V_{inj,1} \) and \( V_{inj,2} \) are the injection volumes used for the two different columns.

The information generated by SEC in combination with a concentration-sensitive detector reflects the molecular-size-distribution (MSD). This distribution is converted into a molar-mass distribution (MMD) using a calibration curve. The MMD is characterized by the characteristic molecular-weight averages (e.g. the number-average molecular-weight, \( M_n \), and the weight-average molecular-weight, \( M_w \)). In SEC the calibration curve plays an essential role. A small variation in the calibration curve may lead to large differences in the characteristic averages. For the determination of the polydispersity (PDI=\( M_w/M_n \)), which is one of the most important properties of polymer samples, large variations in \( M_n \) and \( M_w \) are catastrophic. The SEC calibration curve is usually obtained by injecting a series of well-

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**Table 1:** Dimensions of possible separation columns (\( d_c \) column diameter, \( L \) column length, \( d_p \) particle diameter, \( V_{col} \) column volume)

<table>
<thead>
<tr>
<th>The column</th>
<th>( d_c ) (mm)</th>
<th>( L ) (mm)</th>
<th>( d_p ) ((\mu)m)</th>
<th>( V_{col} ) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard SEC</td>
<td>7.8</td>
<td>500 to 1500</td>
<td>5 to 20</td>
<td>24</td>
</tr>
<tr>
<td>Standard HPLC</td>
<td>4.6</td>
<td>150 to 500</td>
<td>5 to 20</td>
<td>9</td>
</tr>
<tr>
<td>Narrow bore</td>
<td>2.0</td>
<td>150 to 500</td>
<td>3 to 10</td>
<td>1.5</td>
</tr>
<tr>
<td>(\mu)-bore</td>
<td>1.0</td>
<td>150 to 500</td>
<td>3 to 10</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td></td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>Packed capillary</td>
<td>0.2</td>
<td>150 to 500</td>
<td>3 to 10</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td></td>
<td></td>
<td>0.004</td>
</tr>
</tbody>
</table>

* For \( L = 500 \) mm.
characterized standards. The standards should have the same chemical structure as the sample of interest. In this direct-standard-calibration procedure, narrowly distributed (i.e. low-PDI) standards are used. The calibration curves are generally described as polynomial functions (of log MM vs. the retention time, $t_R$, or retention volume, $V_R$). Usually, third-order polynomials are employed to model the calibration curve, but often no motivation is given for the choice of this specific function [24,28]. Vander Heyden et al. [29] demonstrated that in most cases the third-order polynomial is objectively the best choice for describing the calibration curves. The authors used several different chemometric methods to evaluate the shape of the calibration curve [30] based on experimental results [29]. They concluded that six standards were sufficient to generate a reliable calibration curve.

After miniaturization we should obtain the same calibration curves as in conventional SEC. Implicitly the MMD obtained using the miniaturized system should be identical to the MMD obtained by conventional SEC. In this way less mobile phase, less stationary phase, and less sample are needed to obtain the same information. The slow diffusion of macromolecules, however, may lead to increased dispersion in and around μ-bore columns. Due to the dispersion phenomena, it is difficult to obtain identical $M_n$, $M_a$, and peak molecular weight ($M_p$) values. Therefore a statistical treatment using confidence intervals [31] is useful.

In polymer analysis the total band-broadening is due to three different contributions. One is the contribution of the polydispersity of the sample (i.e. the SEC selectivity), which we want to be as large as possible. The other two contributions, column and extra-column dispersion, must be minimized. Previously [32], it has been demonstrated that the dominant contribution to dispersion of narrow standards on single columns (up to 250 mm length) is chromatographic dispersion. This is in agreement with the observations of Lee et al., who demonstrated that narrower peaks (narrower apparent MMDs) could be obtained by temperature-gradient interaction chromatography (TGIC). They explained their results by commenting that chromatographic band-broadening significantly affects the observed peaks in SEC [33,34].

SEC is an imperfect technique, in which band dispersion plays a significant role. Therefore, achieving identical results is nearly impossible. Our ambition for miniaturized SEC is to achieve equivalent results in terms of sample MMDs. Because SEC is imperfect, it is - in principle - possible to achieve better results (more-accurate MMDs) than in conventional
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SEC, especially for narrowly distributed samples. However, more-accurate MMDs are not among the direct advantages of miniaturized SEC and therefore are not the objective of the present study. Indirectly, miniaturization of SEC may contribute to better results, for example by enhancing the compatibility of SEC and MS.

3.3. Experimental

In this study standard HPLC columns 250×6.2 mm *i.d.* and 250×4.6 mm *i.d.* were compared to a narrow-bore column (250×3.0 mm *i.d.*), all manufactured by DuPont (Wilmington, DE USA) and packed with Zorbax PSM (particle diameter 5 μm, pore diameter 100 Å). The effective separation range of these columns is between 10,000 and 1,000,000 Da. This series of experiments will be described in section 3.4.1. The second series of experiments (section 3.4.2) consists of a comparison of a standard HPLC column (250×4.6 mm *i.d.*) with a μ-bore column (250×1.0 mm *i.d.*), both manufactured by Polymer Laboratories (PL) (Church Stretton, Shropshire, UK) and packed with 5-μm Mixed-C material. The effective range of these columns is from 200 to 2,000,000 Da.

Table 2: Narrow polystyrene standards used in the two series of experiments.

<table>
<thead>
<tr>
<th>MM (Da)</th>
<th>Manufacturer</th>
<th>Experiment section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,310</td>
<td>Polymer Laboratories</td>
<td>3.4.2</td>
</tr>
<tr>
<td>10,300</td>
<td>Pressure Chemical</td>
<td>3.4.1</td>
</tr>
<tr>
<td>13,880</td>
<td>Polymer Laboratories</td>
<td>3.4.2</td>
</tr>
<tr>
<td>19,800</td>
<td>Pressure Chemical</td>
<td>3.4.1</td>
</tr>
<tr>
<td>13,880</td>
<td>Polymer Laboratories</td>
<td>3.4.2</td>
</tr>
<tr>
<td>20,400</td>
<td>Pressure Chemical</td>
<td>3.4.1</td>
</tr>
<tr>
<td>51,000</td>
<td>Pressure Chemical</td>
<td>3.4.1</td>
</tr>
<tr>
<td>126,000</td>
<td>Polymer Laboratories</td>
<td>3.4.2</td>
</tr>
<tr>
<td>160,000</td>
<td>Pressure Chemical</td>
<td>3.4.1</td>
</tr>
<tr>
<td>411,000</td>
<td>Pressure Chemical</td>
<td>3.4.1</td>
</tr>
<tr>
<td>523,000</td>
<td>Polymer Laboratories</td>
<td>3.4.2</td>
</tr>
<tr>
<td>670,000</td>
<td>Pressure Chemical</td>
<td>3.4.1</td>
</tr>
<tr>
<td>860,000</td>
<td>Pressure Chemical</td>
<td>3.4.1</td>
</tr>
<tr>
<td>1,112,000</td>
<td>Polymer Laboratories</td>
<td>3.4.2</td>
</tr>
</tbody>
</table>

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The standards used to characterize the columns were narrowly distributed polystyrenes from various manufacturers (Table 2). A broad sample of 271,000 Da and PDI=2.1 from BHD Chemicals (Poole, UK) was used to compare the series of columns. The sample solutions had concentrations of approximately 1 mg/ml in tetrahydrofuran (THF; Biosolve, Valkenswaard, The Netherlands). Toluene (HPLC-grade from Rathburn Chemicals, Walkeburn, Scotland) was used as total-permeation marker.

A Shimadzu (Kyoto, Japan) LC-10ADVP solvent-delivery module and an automated injection-switching valve from VICI (Valco Instruments, Schenkon, Switzerland) were used. The analytes were detected with a UV detector, model 200 from Linear Instruments (Reno, NV, USA). The UV detector was maintained at a wavelength of 260 nm. The detector cell was miniaturized by installing a fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with an internal diameter of 250 μm from the column, through the detector, to waste. Detection was performed through a window in the capillary, from which the coating was removed.

The purpose of the experiment was to study miniaturization of SEC. For this purpose the broad standard (MM=271,000 Da and PDI=2.1) was analyzed on the five different separation columns. The experimental conditions are presented in Table 3.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Column dimensions (mm i.d.)</th>
<th>Experiment Section</th>
<th>Flow rate (ml/min)</th>
<th>Injection volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DuPont</td>
<td>250x6.2</td>
<td>3.4.1</td>
<td>1.0</td>
<td>40</td>
</tr>
<tr>
<td>DuPont</td>
<td>250x4.6</td>
<td>3.4.1</td>
<td>0.55</td>
<td>22</td>
</tr>
<tr>
<td>DuPont</td>
<td>250x3.0</td>
<td>3.4.1</td>
<td>0.22</td>
<td>10</td>
</tr>
<tr>
<td>Polymer Laboratories</td>
<td>250x4.6</td>
<td>3.4.2</td>
<td>0.55</td>
<td>22</td>
</tr>
<tr>
<td>Polymer Laboratories</td>
<td>250x1.0</td>
<td>3.4.2</td>
<td>0.026</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3: Experimental conditions used in the present study.
3.4. Results and discussion

3.4.1. Comparison of standard and narrow-bore columns

If we are to benefit from the advantages and the potential of miniaturized SEC, then characterization of the broad PS standard should yield equivalent information in μ-SEC and in conventional SEC (largest column volumes). Calibration curves were created to obtain the MMD of the sample of interest. Figure 2 shows a typical comparison of three calibration curves corresponding to the 250×6.2 mm i.d., 250×4.6 mm i.d. and 250×3.0 mm i.d. separation columns. The injection volumes and flow rates were adapted to the column dimensions (eq. 1 and 2) in order to keep the column loading and the transversal velocities inside the column constant. The resulting MMDs are plotted in Figure 3, while the corresponding averages are presented in Table 4.

![Calibration curves](image_url)

*Figure 2: Calibration curves for 250×6.2, 250×4.6 and 250×3.0 mm i.d. separation columns packed with Zorbax-PSM(5-μm particles, 100-Å pore-sizes). The flow rates and injection volumes were 1 ml/min and 40 μL (250×6.2 mm i.d.); 0.55 ml/min and 22 μL (250×4.6 mm i.d.) and 0.24 ml/min and 10 μL for the (250×3.0 mm i.d.).*
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Figure 3: Comparison of the MMDs obtained for the broad standard (PS, 271,000 Da, PDI=2.1) using three different columns: 250×6.2 mm i.d. (1.0 ml/min flow rate and 40 μL injection volume), 250×4.6 mm i.d. (0.55 ml/min flow rate, and 22 μL injection volume) and 250×3.0 mm i.d. (0.24 ml/min flow rate and 10 μL injection volume).

Table 4: Average molar masses characterizing the obtained MMDs for the broad PS standard of Figure 3. The values specified by the manufacturer are $M_p=271,000$ and PDI=2.1

<table>
<thead>
<tr>
<th>Separation column</th>
<th>250×6.2 mm i.d.</th>
<th>250×4.6 mm i.d.</th>
<th>250×3.0 mm i.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_p$</td>
<td>298,000</td>
<td>295,000</td>
<td>224,000</td>
</tr>
<tr>
<td>$M_w$</td>
<td>255,000</td>
<td>392,000</td>
<td>233,000</td>
</tr>
<tr>
<td>$M_n$</td>
<td>111,000</td>
<td>254,000</td>
<td>110,000</td>
</tr>
<tr>
<td>PDI</td>
<td>2.30</td>
<td>2.20</td>
<td>2.12</td>
</tr>
</tbody>
</table>

An immediate conclusion from Table 4 is that different numbers are obtained when different columns are used. A second conclusion is that larger columns do not necessarily yield better results. The differences between the different columns are not the results of variations in the calibration curves, because these are very similar for the two smaller columns. Yet the shapes of the obtained MMDs and the characteristic averages are different between these three columns. Thus, the quality of the columns and the resulting elution profiles appear to play a greater role than variations in the calibration curves.
3.4.2. **Comparison between standard and micro-bore columns**

The calibration curve obtained on the 250×4.6 mm *i.d.* column is compared to the one obtained on the 250×1.0 mm *i.d.* column in Figure 4. It can be observed that the calibration curves are somewhat different. The (relative) retention volume is found to be larger on the microbore column, suggesting that this column is more loosely packed (more empty space outside the particles). As this difference will be the same for standards and samples, it should not necessarily affect the observed MMDs. However, the MMDs obtained on the two columns also appear to be different (Figure 5). The MMD obtained from the μ-bore column (top curve) is significantly broader and slightly shifted. Comparing the two MMDs (Figure 7) of the broad PS (271,000 Da), obtained using the calibration curves of Figure 4 and the elution profiles of Figure 5, we can obtain the $M_{n}$, $M_{w}$, and $M_{z}$ as in Table 5.

There are – at least in principle – two different ways to explain the difference between the two MMDs, *viz.* errors associated with the calibration and chromatographic dispersion effects. To investigate the possibility of the former explanation, we have performed a statistical analysis of the possible error. In Figure 6 the calibration curves for the two columns are plotted with statistical 95% confidence intervals. Using these as an indication of the possible error in the calibration, we also calculated confidence intervals for the derived MMDs (Figure 7). The different curves from Figure 7 are overlaid in Figure 8. This latter Figure shows that, even though broad confidence intervals are assigned to each MMD, they differ significantly at both extremes (low- and high-MM regions). This clearly demonstrates that the difference cannot be explained by possible errors in the calibration. Significantly more band broadening is observed on the μ-bore column than on the conventional column and this is reflected in an apparently broader MMD.

This is also evident from the data in Table 5. The edges of the broader elution profile obtained on the μ-bore column extend outside the measured part of the calibration curve. To convert the complete elution profile into a complete MMD extrapolation is required. This may cause significant errors (see Table 5b). Restricting the calculated MMD to the statistically relevant part of the calibration curve, however, often meets objectives from polymer scientists and chromatographers. An example can be found in Figure 3.
While it is possible to perform software corrections on the observed elution profiles [35,36], the fundamentally more-correct approach is to improve the efficiency of μ-bore columns and the associated instruments for SEC.

![Figure 4: Calibration curves for 250×4.6 mm i.d. (●) (experimental conditions: flow rate 0.55 ml/min and injection volume 22 μL) and 250×1.0 mm i.d. (●) (experimental conditions: 0.026 ml/min and 1.0 μL) columns. Relative retention times with respect to toluene are used to compare the two curves.](image)

![Figure 5: MMDs for the broad PS sample (271,000 Da, PDI=2.1), obtained using the 250×1.0 mm i.d. column (flow rate 0.026 ml/min, injection volume 1.0 μL, top curve) and the 250×4.6 mm i.d. column (0.55 ml/min and 22 μL, bottom curve).](image)
Figure 6: Confidence intervals for the calibration curves obtained on the 250×4.6 mm i.d. (a) and 250×1.0 mm i.d. (b) columns with the associated confidence intervals.

Figure 7: Confidence intervals calculated for the MMDs of Figure 5 using the possible variations around the calibration curves indicated in Figure 6.
Figure 8: Overlay of the obtained MMDs (Figure 7) illustrating that the difference between the curves cannot be explained by possible variations in the calibration curves. The gray surface represents the 250×4.6 mm i.d and the ‘striped’ area the 250×1.0 mm i.d. The black curve (middle of the gray area) indicates the most probable MMD.

Table 5: Characteristic molar masses and PDI values calculated from the MMD (Figure 7) of a broad PS obtained from the 250×4.6 and 250×1.0 mm i.d. columns. The values given by the manufacturer are: M_p=271,000 and PDI=2.1.

a) with interpolation of the calibration curve (restricted MM range)

<table>
<thead>
<tr>
<th>Column (mm i.d.)</th>
<th>M_p</th>
<th>M_n</th>
<th>M_m</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>250×1.0</td>
<td>382,000</td>
<td>566,000</td>
<td>118,000</td>
<td>2.92</td>
</tr>
<tr>
<td>250×4.6</td>
<td>281,000</td>
<td>270,000</td>
<td>114,000</td>
<td>2.38</td>
</tr>
</tbody>
</table>

b) with extrapolation of the calibration curve (complete MM range)

<table>
<thead>
<tr>
<th>Column (mm i.d.)</th>
<th>M_p</th>
<th>M_n</th>
<th>M_m</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>250×1.0</td>
<td>382,000</td>
<td>517,000</td>
<td>132,000</td>
<td>3.91</td>
</tr>
<tr>
<td>250×4.6</td>
<td>281,000</td>
<td>277,000</td>
<td>114,000</td>
<td>2.42</td>
</tr>
</tbody>
</table>

3.5. Conclusions – state of the art

Miniaturized SEC is possible and it can be applied in a number of situations. Reducing the column diameters from conventional (about) 8 to 10 mm to the more modern range of 3 to 5 mm is reasonably possible. However, band broadening does play a significant role in
SEC, especially for narrowly distributed samples. Therefore, different columns are likely to yield considerably different, yet statistically equivalent characteristic averages \((M_n, M_w, PDI)\).

The use of microbore columns (1 mm \(i.d\)) in SEC is still quite difficult. Suitable instruments, especially detectors, are not available. Even more important is the lack of good columns. The present research and preliminary investigations [37] have been conducted over a period of nearly ten years. Throughout this period, we have consistently experienced poor efficiency on 1-mm SEC columns, despite genuine efforts by some of the best commercial companies and some of the best researchers. Truly miniaturized SEC thus remains a great challenge.

During this period in time, the motivators for research in SEC have also changed. The perceived advantages of miniaturization pertain (less solvent, sample, and stationary phase needed, less waste produced, etc.), but so do the obstacles (no columns, no instruments, no detectors). During the same period, interest in Fast SEC has increased dramatically. Fast SEC has important, but not necessarily overriding advantages in the overlapping fields of combinatorial chemistry and high-throughput experimentation. However, the greatest driver for both miniaturized SEC and Fast SEC may come from within analytical chemistry. The interest in multi-dimensional separations has increased tremendously in recent years and comprehensive two-dimensional liquid chromatography is increasingly applied to large biomolecules and synthetic polymers. SEC is potentially extremely useful as one stage in a multi-dimensional separation system, because it separates on the basis of molecular size, whereas other techniques (interactive LC, ion-exchange LC) exploit quite different mechanisms. In comprehensive two-dimensional liquid chromatography, SEC can be used either as a first-dimension or as a second-dimension technique (SEC×LC or LC×SEC, respectively). In the former case miniaturization (down to around the awkward column \(i.d\) of 1 mm) is recommended [38]. In the latter case high speed is vital. Earlier the advantages of LC×SEC had been weighted greater than those of SEC×LC [38]. Thus, there is a greater motivation to perform Fast SEC than to develop small SEC. In turn, successes in the development of Fast SEC (see Chapters 2, 4, 5) contribute to the advantages of LC×SEC, whereas obstacles encountered in miniaturizing SEC add to the disadvantages of SEC×LC.
3.6. Acknowledgement

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References

Chapter 3